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Influence Breast Cancer Cell Proliferation and
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INTRODUCTION

Accumulating evidence suggests that monocytes and macrophages are recruited to tumors where, in response to microenvironmental stimuli, they secrete inflammatory products, growth factors, and angiogenic cytokines that may promote tumor growth and metastasis (1). Macrophages may constitute as much as half of the mass of cells in some tumors, including breast tumors, and their presence has been shown to correlate with a poor prognosis (2). The critical role of monocytes and macrophages in angiogenesis has been exemplified by the identification of thymidine phosphorylase (TP), a known angiogenic factor, as a monocyte or macrophage product. TP has long been associated with the propensity for angiogenic growth, but its mechanism of action has been elusive. Recently, it was shown that a dephosphorylated product of the TP reaction, 2-deoxyribose (2-dR), is a chemoattractant for vascular endothelial cells (EC) (3). Tumor-associated macrophages produce 2-dR which recruits vascular ECs; under the influence of stromal cell-derived cytokines, these ECs form a tumor vasculature. Macrophage involvement in tumor initiation and progression is further supported by the identification of the macrophage scavenger receptor (MSR)-1 gene as one of two prostate cancer susceptibility genes (4); by the identification of tumor susceptibility genes that are macrophage-associated risk inflammatory factors (5); and by the inclusion of *CD68*, another macrophage scavenger receptor gene, in the Genomic Health Oncotype DX breast cancer assay developed by Genomic Health (6). Taken together, these observations suggest that inflammation is a driving force in tumorigenesis, and that the monocyte and macrophage are critical effectors in the establishment and maintenance of a tumor-inducing stroma. Tumor growth depends on angiogenesis and is a precursor of metastasis. It may be possible to suppress both angiogenesis and metastasis by inhibiting the inflammatory activities of macrophages with anti-inflammatory drugs. Our preliminary studies show that MCF-7 breast cancer cells can skew the transcriptional profile of THP-1 macrophages toward the expression of angiogenesis-related genes (8). These findings suggest that the ability of tumor cells to modulate macrophage gene expression may determine their angiogenic and metastatic potential. In the tumor microenvironment, macrophages are known to secrete cytokines which can drive tumor progression via their effects on angiogenesis, invasion, and metastasis, as well as on tumor immunity. The interaction between tumor cells and stromal cells is dynamic and transactional, and several variables, including the tissue-specific phenotype of the macrophages, contact time, the stability of the changes induced in tumor cells, and the potential of pharmacological agents to reverse these changes, are yet to be determined. Our studies focus on the interactions between macrophages and tumor cells, rather than the cells themselves, as targets of therapeutic intervention. Our results suggest that *in vitro* assays of anticancer agents should be conducted on tumor cells in the presence of stromal cells that play a role in modulating tumor phenotype.

BODY

STATEMENT OF WORK

Tamoxifen and tumor-associated macrophages

Task 1. Determine the effect of *in vitro* co-culture on gene expression in BC cells and THP-1-macrophages (Months 1 - 18):

- (a) Recruitment of postdoctoral fellow (Months 1 - 2)
- (b) Grow cells, set up co-cultures of BC and THP-1 macrophages (Months 1 - 7)
- (c) Isolate mRNA for gene expression array analysis, Months 4 - 12
- (d) Standardize and calibrate gene expression arrays for proliferation-related gene expression in

BC cells co-cultured with THP-1 macrophages (Months 11 - 18)

Task 2. Studies on effects of anti-inflammatory and macrophage-modulating compounds on macrophage and BC gene expression (Months 16 - 36):

- (a) Co-culture of cells for studies on the effects of anti-inflammatory agents (Months 16 - 30)
- (b) Isolation of mRNA for RT-PCR, and gene expression arrays for Task 2 (Months 17- 30)
- (c) Western blotting, ELISA for cytokines, RT-PCR (Months 24 - 36)

This report covers activities included in *Task 1(d)* and *Task 2(a)*. Results of *Task 1(d)* were presented in the 2003 annual report and at the American Association for Cancer Research-National Cancer Institute-European Organization for Research and Treatment of Cancer International Conference: *Molecular Targets and Cancer Therapeutics*, Boston, MA, November 17 – 21, 2003 (Reference 8, and Appendix 1). Some results reported in this report were also included in a poster presentation at the 2004 annual meeting of the American Association for Cancer Research in Orlando, FL (9) and Appendix 3.

Angiogenesis is critical for tumor growth. The establishment of a tumor vasculature allows a tumor to grow to a size greater than 1 or 2 mm in diameter. Beyond this size, tumors outstrip the supply of oxygen and nutrients and will die unless a vasculature is established. An increase in tumor mass is a precursor for all of the other stages in tumor progression. Because tumor cells tend to be genetically unstable owing to loss or epigenetic modification of tumor suppressor genes, the larger the number of cells in a tumor the greater is the probability that escape mutants with more aggressive phenotypes will occur and that clonal outgrowth of such cells will lead to invasion and metastasis. Therefore, angiogenic cytokines produced by tumor cells or stromal cells may drive tumor progression. Many cytokines, such as acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), interleukin-8 (IL-8), and vascular endothelial growth factor (VEGF), stimulate both proliferation and angiogenesis.

We have investigated the ability of macrophages to influence angiogenesis related gene expression in MCF-7 breast cancer cells and, reciprocally, the ability of MCF-7 cells to alter THP-1 macrophage gene expression. We have also examined the ability of tamoxifen (TMX), a widely used drug for the prevention and treatment of breast cancer, to modulate this transactional regulation of gene expression between macrophages and breast cancer cells. Appendix 3 shows the lay-out of angiogenesis-related genes in the GE Array Q Series Human Angiogenesis Gene Array (SuperArray, Frederick, MD) used in these studies. We found that bryostatin 1-differentiated THP-1 macrophages express very low levels of IL-8 mRNA. (The use of bryostatin-1 as a differentiating agent in place of phorbol 12-myristate 13-acetate (PMA) is explained below). However, co-culture with MCF-7 dramatically up-regulated *IL-8* under normoxia (Fig. 1A and 1D) but not under hypoxia (Fig. 2), and this up-regulation was not affected by tamoxifen (10 μ M) or aspirin (1 mM) (Fig. 1E & 1F). Unexpectedly, TMX induced *HIF-1 α* expression (Fig. 1B), while aspirin dramatically up-regulated *IL-10* (Fig. 1C). THP-1 macrophages did not express IL-10 under normoxia or hypoxia (F.g. 1A & 2A). However, aspirin up-regulated IL-10 in the macrophages under both normoxia and hypoxia, except when MCF-7 cells are present. We surmise that MCF-7 cells secrete a factor that overrides the ability of aspirin to induce IL-10 in the macrophages. IL-10 is a potent anti-inflammatory cytokine: therefore, the MCF-7-mediated suppression of aspirin-induced *IL-10* expression is consistent

with the ability of tumor cells to maintain a pro-inflammatory microenvironment. By suppressing *IL-10* expression, MCF-7 cells control gene expression in the co-cultured macrophages in a manner that perpetuates a pro-inflammatory environment. A search of PubMed failed to show any report on aspirin regulation of *IL-10* gene expression in macrophages. The ability of aspirin to induce *IL-10* expression may be an additional mechanism by which this widely used non-steroidal anti-inflammatory drug (NSAID) exerts its anti-inflammatory effects. Aspirin (1 mM) also up-regulated *IL-10* in MCF-7 cells, under both normoxia and hypoxia (Fig. 3C & 4C). MCF-7 cells did not express *IL-10*, except when they were treated with aspirin (Fig. 3C & 3D & 4C & 4D). Co-culture of MCF-7 with macrophages or treatment with TMX did not induce MCF-7 *IL-10* under normoxia or hypoxia. However, aspirin induced *IL-10* under both conditions (Fig 5C & 5D). Therefore, aspirin consistently induced *IL-10* expression in both tumor cells and macrophages.

We have also investigated the ability of THP-1 macrophages to influence the proliferation and survival of MCF-7 cells treated with 10 μ M TMX. Fig. 7 shows the survival of adherent MCF-7 cells as measured by the MTT assay in the presence and absence of co-cultured macrophages. The MTT assay was conducted after 3 d of co-culture. Under both normoxia and hypoxia, MCF-7 cells proliferated more rapidly and showed a greater survival rate when they were co-cultured with macrophages. These results are consistent with the hypothesis that tumor-associated macrophages secrete cytokines and other factors that promote tumor cell growth. Next, we tested the ability of aspirin to modulate the macrophage-mediated protection of MCF-7 cells from tamoxifen killing. When THP-1 macrophages were pre-treated with 1 mM aspirin, and then co-cultured with MCF-7 cells that were exposed to varying concentrations of TMX, aspirin treatment of the macrophages completely abrogated the protection of MCF-7 cells from TMX killing (Fig. 8). The large difference in proliferation/survival between MCF-7 cells grown in the presence of macrophages and those grown without macrophages, or with aspirin-treated macrophages, reflects the contribution of the macrophages to MCF-7 proliferation over 3 days of co-culture.

During the course of these investigations, we learned that PMA, the reagent used to differentiate THP-1 monocytes to macrophages, also induced *IL-8* expression in several cell types. Therefore, we could not be sure whether the high expression of *IL-8* observed in PMA-differentiated macrophages was due to their status as differentiated macrophages or to PMA stimulation. Accordingly, we used another macrophage-differentiating agent, bryostatin 1, at a concentration of 10 nM, to differentiate THP-1 monocytes. Bryostatin 1 is not known to induce *IL-8* expression, and *IL-8* expression was negligible in bryostatin 1-differentiated THP-1 macrophages. All subsequent studies, including the studies reported herein, were done with bryostatin 1-differentiated THP-1 macrophages.

RESEARCH ACCOMPLISHMENTS

- We have found that aspirin (1 mM) induces the expression of *IL-10*, a potent anti-inflammatory cytokine gene in both macrophages and breast cancer cells, suggesting a novel mechanism for the anti-inflammatory action of aspirin.
- We have shown that MCF-7 breast cancer cells suppress *IL-10* expression in co-cultured macrophages, even when the cells are treated with aspirin, suggesting that MCF-7 cells secrete a factor that overrides the ability of aspirin to induce *IL-10*.

- We have shown that co-cultured macrophages protect MCF-7 cells from tamoxifen killing, suggesting that tumor-infiltrating macrophages may attenuate the effects of tamoxifen therapy.
- We have shown that aspirin abrogates the protection of MCF-7 cells against tamoxifen killing conferred by macrophages, providing a rationale for an adjuvant role of aspirin in combination tamoxifen therapy.
- We have formulated a testable hypothesis that IL-10 is a critical mediator of tumor cell-stromal cell interaction, and that inflammation promotes rather than protects against tumor growth, as has been suggested by others. This development was not envisioned in the original proposal. The role of IL-10 in tumor growth can be investigated by ablation of IL-10 with anti-IL-10 antibody or through RNA interference.

REPORTABLE OUTCOMES

1. Morris, G. S. and **Bremner, T. A.** (2003). Tamoxifen alters the inflammatory cytokine transcriptional profile induced in THP-1 macrophages by MCF-7 breast cancer cells. *Proceedings of the AACR-NCI-EORTC International Conference : Molecular Targets and Cancer Therapeutics*, Boston, MA, November 17 – 21, 2003 (**ABSTRACT B197**). Poster presentation. Copy of abstract in Appendix 1.
2. Morris, G. S., Henry, G. A., and **Bremner, T. A.** THP-1 macrophages stimulate proliferation, protect against tamoxifen killing, and modulate angiogenesis-related gene expression in MCF-7 breast cancer cells. *AACR 94th Annual Meeting Proceedings*, vol. 45, 1199. **ABSTRACT #5201**, March 2004. Poster presentation. Copy of abstract in Appendix 2.
3. Ms. Gay Morris, graduate student/technician supported by this grant has completed her Ph.D. dissertation, and will defend same before August 16, 2004. She has accepted a postdoctoral position in the laboratory of Dr. Kent Osborne, Baylor College of Medicine, Houston, TX, where she will receive additional training in breast cancer.
4. Zhe Jin, M.D., Ph.D. joined the laboratory in January 2004 to fill the post-doctoral position on the grant. Dr. Jin was recruited from the Department of Chemical Biology, Susan Lehman Cullman Laboratory for Cancer Research, Ernest Mario School of Pharmacy, Rutgers, The State University of NJ. Dr. Jin earned the M.D. degree from China Medical University (P. R. China) in 1992, and the Ph.D. in Medical Science (Pathology) from Yamagata University School of Medicine (Japan) in 2002.

CONCLUSIONS

The angiogenic switch marks a critical juncture in tumor progression. The molecular changes that drive the development of a tumor vasculature are triggered by signals that originate in both tumor cells and surrounding stromal cells. While stromal and extracellular matrix constituents can exert potent tumor-suppressive effects, investigation of the interplay between tumor cells and stromal cells reveals that tumor cells can reprogram the transcriptome of stromal cells, especially macrophages, to produce a cytokine milieu that promotes cancer cell survival and angiogenesis. The significance of these findings for cancer therapy is profound. This emerging paradigm of

tumor progression implies that therapeutic targets of progression should be sought not only in the transformed epithelial cells themselves but in the stromal cells as well and, more importantly, in the reciprocal signaling that characterizes epithelial-stromal interactions. These are difficult (moving) targets, but they are tractable and hopefully 'drugable'. Our data show that macrophages can protect tumor cells against tamoxifen killing, and that aspirin can abrogate this protection. Recent reports suggest that aspirin and other NSAIDs are effective chemopreventive agents for breast cancer, especially estrogen receptor-positive breast cancer (9). Our data elucidate a novel mechanism for aspirin action in breast cancer chemoprevention and chemotherapy and suggest that tamoxifen in combination with aspirin may be more effective than tamoxifen alone. We are currently confirming the gene array results with RT-PCR and Western blot analysis. However, in cases where both cell types secrete the same cytokine, it would not be possible to determine the contribution of each by measuring the concentration of the cytokine in the medium. Therefore, emphasis is placed on the regulation of gene expression as assessed by changes in mRNA levels.

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APPENDIX 1

Tamoxifen alters the inflammatory cytokine transcriptional profile induced in THP-1 macrophages by MCF-7 breast cancer cells.

Gay S. Morris, Theodore A. Bremner, Howard University, Washington, DC

Tamoxifen (TMX) is the most widely used anti-estrogen for breast cancer prevention and treatment, but its effectiveness is limited by the inevitable development of cellular resistance. The mechanisms that underlie tamoxifen action and resistance are not completely understood. Abundant evidence suggests that stromal cells, including macrophages, promote tumor progression. Therefore, the genetic events that underlie progression may occur in both tumor cells and stromal cells. However, studies of TMX action have generally involved tumor cells only, and not stromal cells. We have used co-cultures of MCF-7 breast cancer cells and THP-1 macrophages to study interactions between macrophages and tumor cells in a simulated tumor environment, and to determine the effects of TMX on the transcriptional profiles of both cell types. MCF-7 cells and THP-1 macrophages were cultured separately or co-cultured with or without 10 μ M TMX. Total RNA was reversed transcribed, biotin-labeled, and hybridized to 96-gene arrays for inflammatory cytokines/receptors. Our results show that MCF-7 cells altered the inflammatory cytokine profile of THP-1 macrophages. Regulated genes included *IL1 β* , *TGF β 1*, and *SCYA20* (MIP-3 α), *SCYA3* (MIP-1 α), *SCYA4* (MIP-1 β), and *SCYA5* (CCL5/RANTES), which were dramatically up-regulated, and *SCYA1* (CCL1), *SCYB13* (CXCL13) and *SCYA23* (CCL23/MIP1-1) which were down-regulated. In contrast, however, when MCF-7 cells were treated with TMX prior to co-culture, *IL1 β* expression in the macrophages was decreased, *SCYA20* and *TGF β 1* expression was lost, but *TGF β 3* and *SCYB13* were up-regulated. However, *SCYA17* (CCL17/TARC), *SCYA22* (CCL22/MDC), *SCYA23*, *SCYA25* (CCL25/TECK), and *SCYB12* (SDF-1 α) were induced. Clearly, TMX modulates the ability of MCF-7 cells to regulate inflammatory cytokine gene expression in THP-1 macrophages. The ability of TMX-treated MCF-7 to up-regulate *TGF β 3* expression in THP-1 macrophages deserves further study. Both IL-1 β and TGF β are implicated in tumor cell survival and metastasis: IL-1 β activates NF- κ B, a known suppressor of apoptosis, and elevated levels of TGF β s have been associated with a more metastatic phenotype in breast cancer. Taken together, these findings suggest that the effectiveness of TMX may be enhanced in combination with chemotherapeutic agents that ablate IL-1 β and TGF β production by intratumoral macrophages. Sponsored by the U.S Army Medical Research and Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014, Award NO: DAMD17-02-1-0408 to Theodore Bremner (PI). The content of this report does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

Keywords: Cytokines; breast cancer; macrophage; tamoxifen

Abstract Category: 11. Metastasis and invasion targets (e.g., MMP inhibitors, adhesion)

APPENDIX 2

THP-1 macrophages stimulate proliferation, protect against tamoxifen killing, and modulate angiogenesis-related gene expression in MCF-7 breast cancer cells

Gay S. Morris, Georgia A.-M. Henry, and Theodore A. Bremner, Howard University, Washington, DC

Stromal cells influence tumor cell proliferation and tumor progression. The emergence of aggressive phenotypes may involve signals extrinsic to cancer cells that are generated in the interaction between cancer cells and stromal cells. We examined breast cancer cell-macrophage interactions in a simulated tumor environment, using co-cultures of THP-1 macrophages (M ϕ) and MCF-7 breast cancer cells. We hypothesized that (1) M ϕ , in the tumor context, may secrete factors that promote tumor cell growth and drug resistance, and (2) tamoxifen (TMX) may stimulate M ϕ production of pro-angiogenic or pro-metastatic factors despite its ability to inhibit tumor cell proliferation. MCF-7 cells and THP-1 M ϕ were co-cultured in varying concentrations of tamoxifen (0 – 15 μ M) for 3 d under normoxia or hypoxia (94% N₂, 1% O₂, and 5% CO₂). MCF-7 cell proliferation and survival were measured by the MTT assay. For gene expression analysis, total RNA was extracted from M ϕ and MCF-7 cells, labeled, and hybridized to gene arrays. Up-regulation of candidate genes was confirmed by RT-PCR. Apoptotic killing of MCF-7 by TMX was assessed by DNA laddering. Under normoxia, MCF-7 cells proliferated more rapidly when co-cultured with THP-1 M ϕ . In the absence of M ϕ , only 17% of MCF-7 cells survived 15 μ M TMX, whereas in the presence of M ϕ , survival was 72%. Under hypoxia, survival in the absence of M ϕ was 9%, and 55% in the presence of M ϕ . These data suggest that M ϕ reduced the susceptibility of MCF-7 cells to TMX killing. The most dramatic differences in growth factor- and angiogenesis-related gene expression were observed between MCF-7 cells alone and MCF-7 in co-culture, under hypoxia. Therefore, the presence of M ϕ modulated gene expression in MCF-7 cells. Specifically, in TMX-treated (10 μ M) co-cultures, *VEGF*, *VEGF-D*, *osteopontin*, *thrombospondin*, and *HIF-1 α* were all up-regulated in MCF-7 cells under hypoxia. Additionally, M ϕ induced the expression of *IL-8*, *MDK*, *T β Rs*, 1, 2, 3, and *ID3*. Our findings suggest that the killing efficiency of TMX may be increased by its combination with drugs that suppress M ϕ control of angiogenesis-related gene expression. A more extensive study of the effects of TMX on the interaction between M ϕ and tumor cells may provide valuable information for the development of combination therapies directed at both tumor cells and tumor-associated M ϕ . Sponsored by the U.S Army Medical Research and Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014, Award NO: DAMD17-02-1-0408 to Theodore Bremner (PI). The content of this report does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

APPENDIX 3

GEArray Q Series Human Angiogenesis Gene Array (HS-009)

Array Layout

ADAMTS1 1	ADAMTS8 2	RNASE4 3	ANGPT1 4	ANGPT2 5	CD36 6	CDH5 7	CHGA 8
CSF3 9	EDG1 10	EFNA2 11	EFNA5 12	EFNB2 13	EGF 14	EGFR 15	ENG 16
EPHB4 17	ERBB2 18	ETS1 19	F2 20	FGF1 21	FGF2 22	FGF4 23	FGF6 24
FGF7 25	FGFR1 26	FGFR2 27	FGFR3 28	FGFR4 29	FIGF 30	KDR 31	FLT1 32
FN1 33	CXCL1 34	HGF 35	HIF1A 36	HPSE 37	ID1 38	ID3 39	IFNA1 40
IFNB1 41	IFNG 42	IGF1 43	IL10 44	IL12A 45	IL8 46	ITGA5 47	ITGAV 48
ITGB3 49	AMOT 50	COL18A1 51	SMAD1 52	MDK 53	MMP2 54	MMP9 55	MSR1 56
NOS3 57	NRP1 58	PDGFA 59	PDGFB 60	PDGFRA 61	PDGFRB 62	PECAM1 63	PF4 64
PGF 65	PLAU 66	PRL 67	PTGS1 68	PTGS2 69	PTN 70	RSN 71	CCL2 72
SERPINB5 73	SERPINF1 74	SPARC 75	SPP1 76	TEK 77	TGFA 78	TGFB1 79	TGFB2 80
TGFB3 81	TGFBR1 82	TGFBR2 83	TGFBR3 84	THBS1 85	THBS2 86	THBS3 87	THBS4 88
TIE 89	TIMP1 90	TIMP2 91	TNF 92	TNFSF15 93	VEGF 94	VEGFB 95	VEGFC 96
PUC18 97	PUC18 98	PUC18 99	Blank 100	Blank 101	Blank 102	GAPD 103	GAPD 104
PPIA 105	PPIA 106	PPIA 107	PPIA 108	RPL13A 109	RPL13A 110	ACTB 111	ACTB 112

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APPENDIX 4 (Figs 1 – 8)

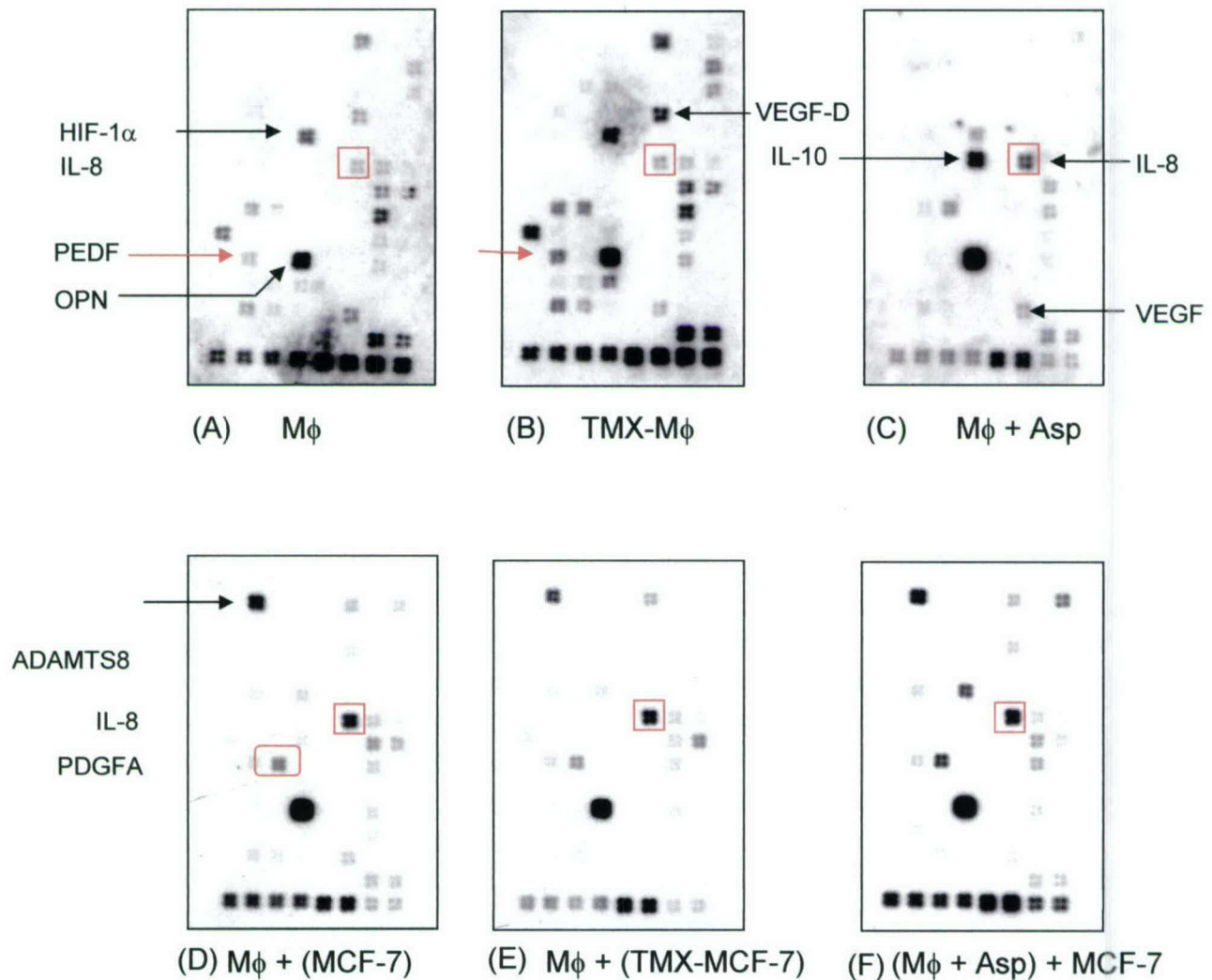


Fig. 1: Angiogenesis-related gene expression in macrophages under normoxia. Mφ, macrophages, TMX, tamoxifen, Asp, aspirin. Bryostatin 1-differentiated macrophages and MCF-7 cells were co-cultured in the upper and lower wells, respectively, of Costar Transwell™ chambers. The cells were separated by a membrane of pore size 3 μm, which allowed passage of diffusible molecules, but not cells. After co-culture, mRNA was extracted and used for gene arrays.

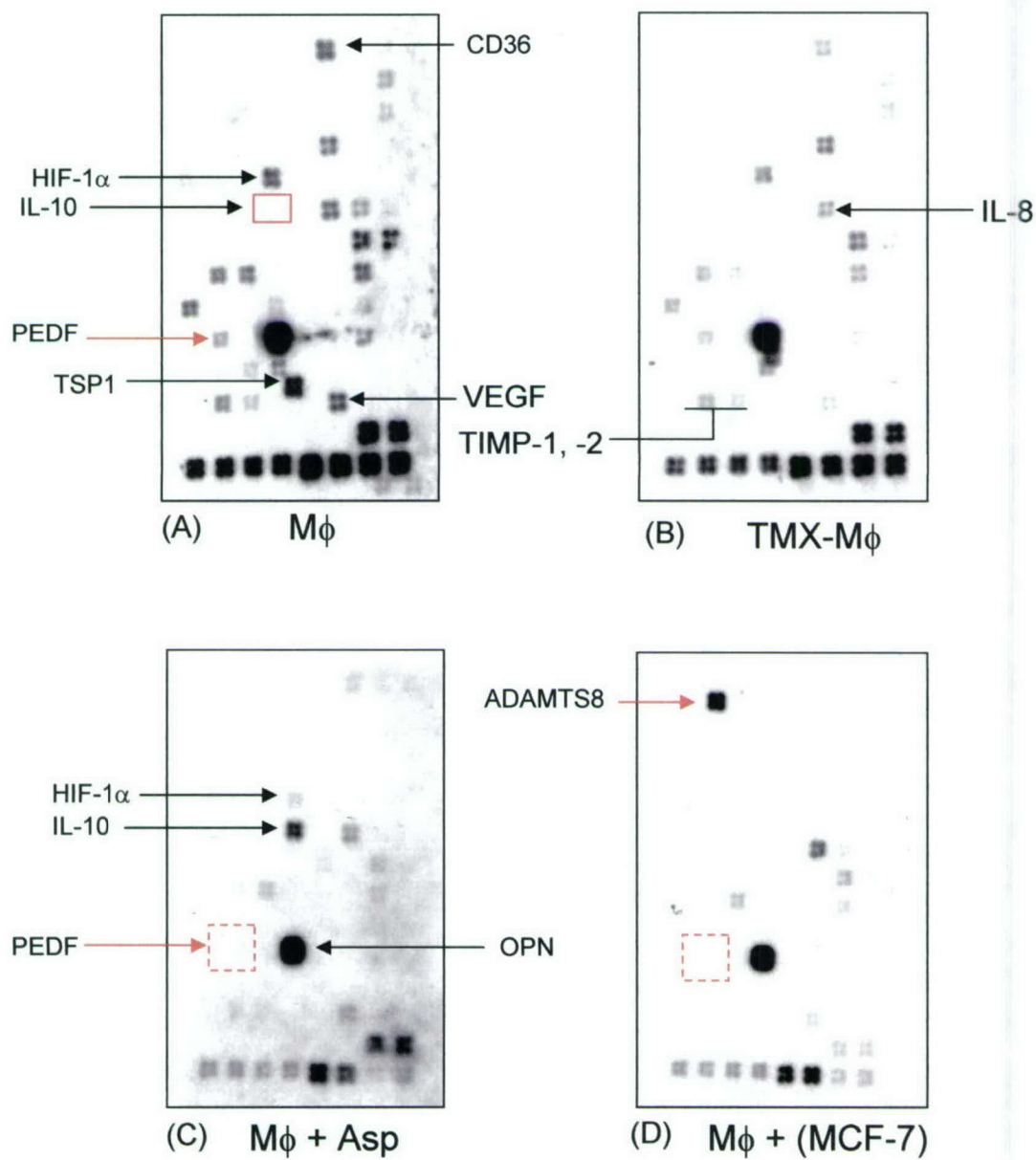


Fig. 2: Angiogenesis-related gene expression in macrophages under hypoxia. Mφ, macrophage; TMX, tamoxifen; Asp, aspirin. Methods same as for Fig 1.

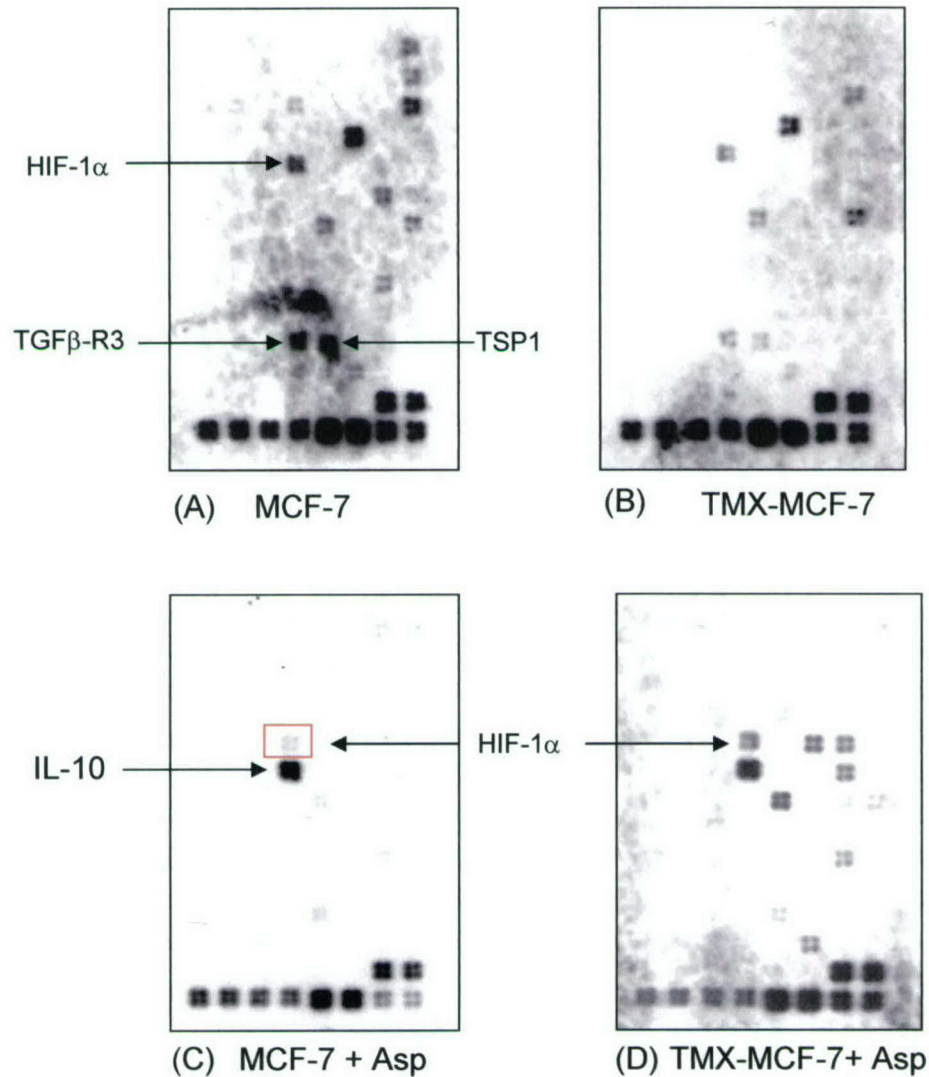


Fig. 3: Angiogenesis-related gene expression in MCF-7 cells under normoxia. M ϕ , macrophages, TMX, tamoxifen, Asp, aspirin. Bryostatin 1-differentiated macrophages and MCF-7 cells were co-cultured in the upper and lower wells, respectively, of Costar TranswellTM chambers. The cells were separated by a membrane of pore size 3 μ m, which allowed passage of diffusible molecules, but not cells. After co-culture, mRNA was extracted and used for gene arrays.

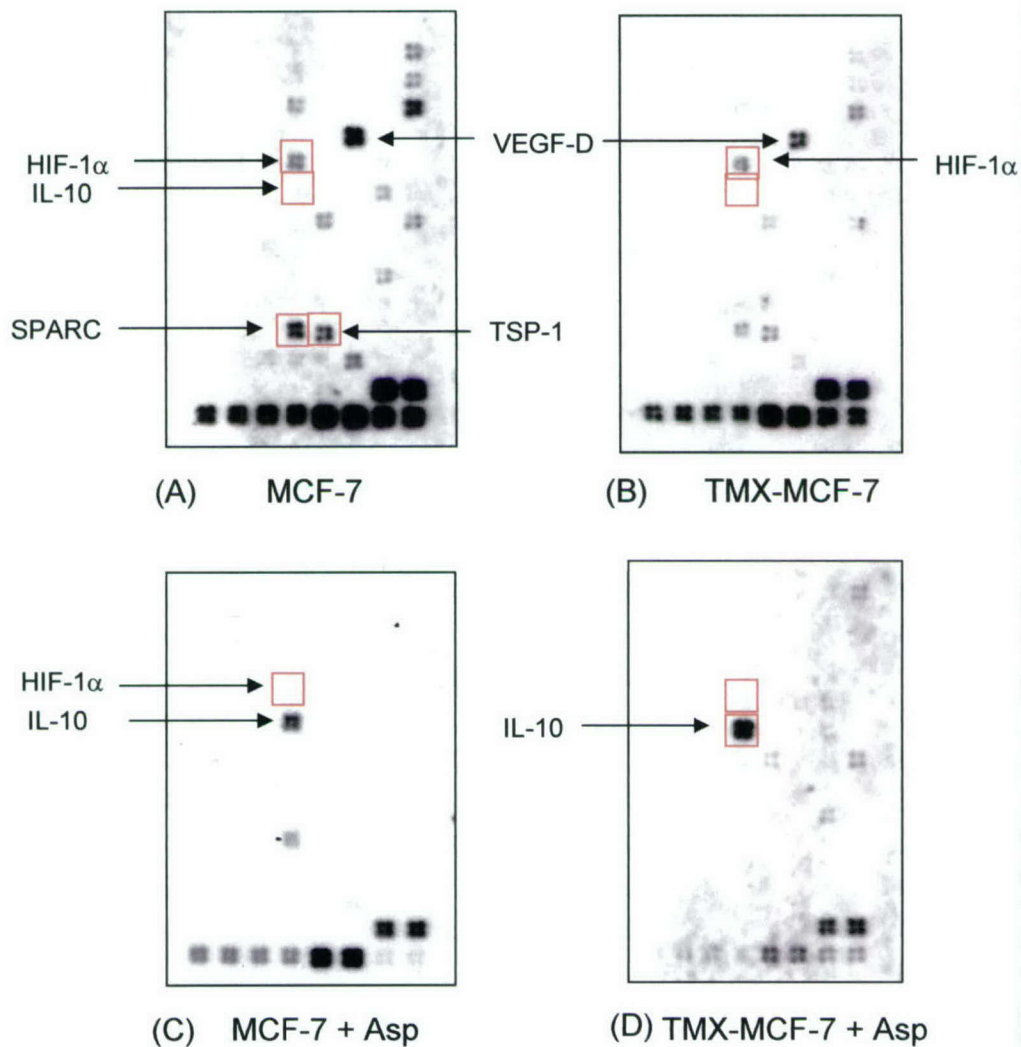


Fig. 4: Angiogenesis-related gene expression in MCF-7 cells under hypoxia. M ϕ , macrophages, TMX, tamoxifen, Asp, aspirin. Bryostatin 1-differentiated macrophages and MCF-7 cells were co-cultured in the upper and lower wells, respectively, of Costar Transwell™ chambers. The cells were separated by a membrane of pore size 3 μ m, which allowed passage of diffusible molecules, but not cells. After co-culture, mRNA was extracted and used for gene arrays.

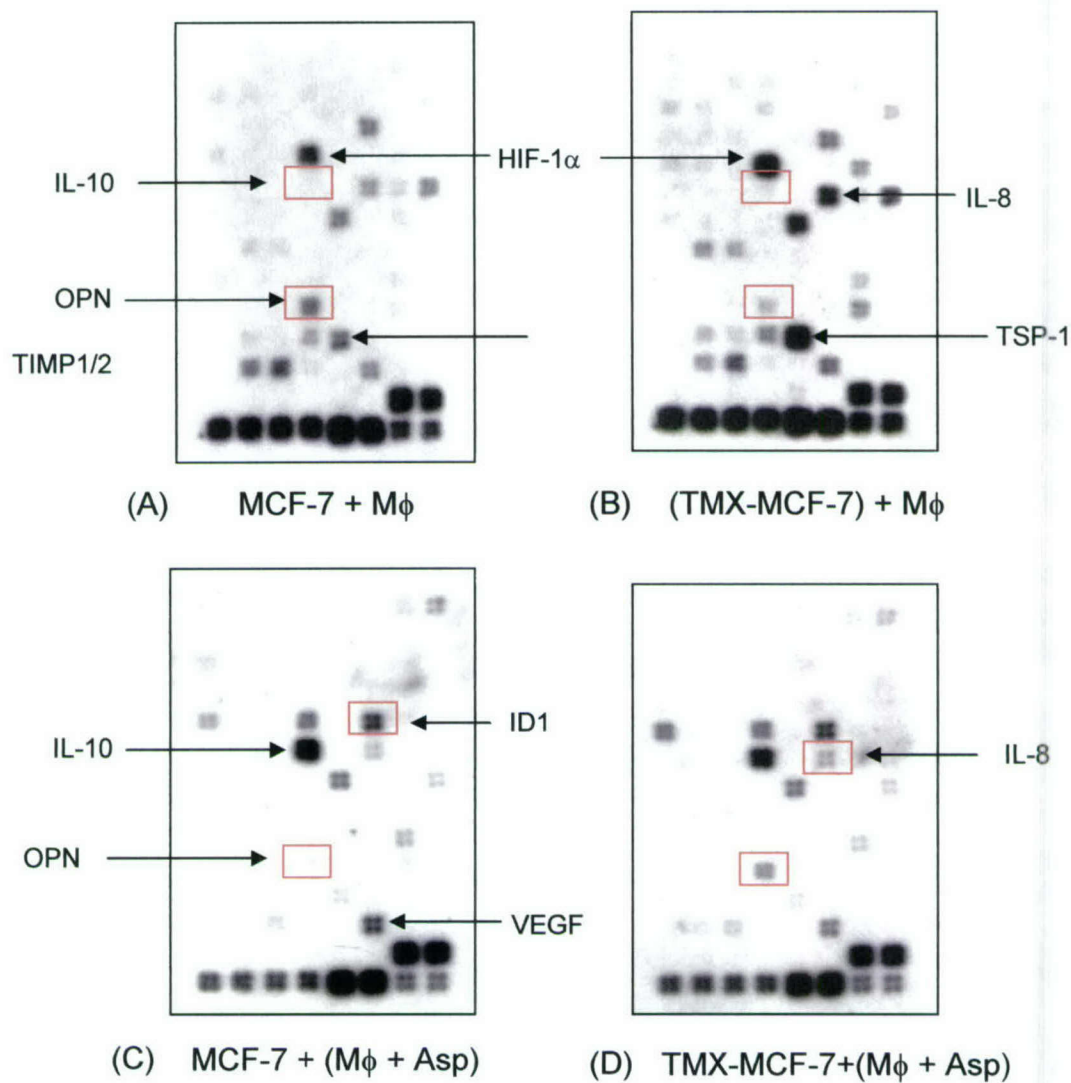


Fig. 5: Angiogenesis-related gene expression in MCF-7 cells under normoxia. M ϕ , macrophages, TMX, tamoxifen, Asp, aspirin. Bryostatin 1-differentiated macrophages and MCF-7 cells were co-cultured in the upper and lower wells, respectively, of Costar Transwell™ chambers. The cells were separated by a membrane of pore size 3 μ m, which allowed passage of diffusible molecules, but not cells. After co-culture, mRNA was extracted and used for gene arrays.

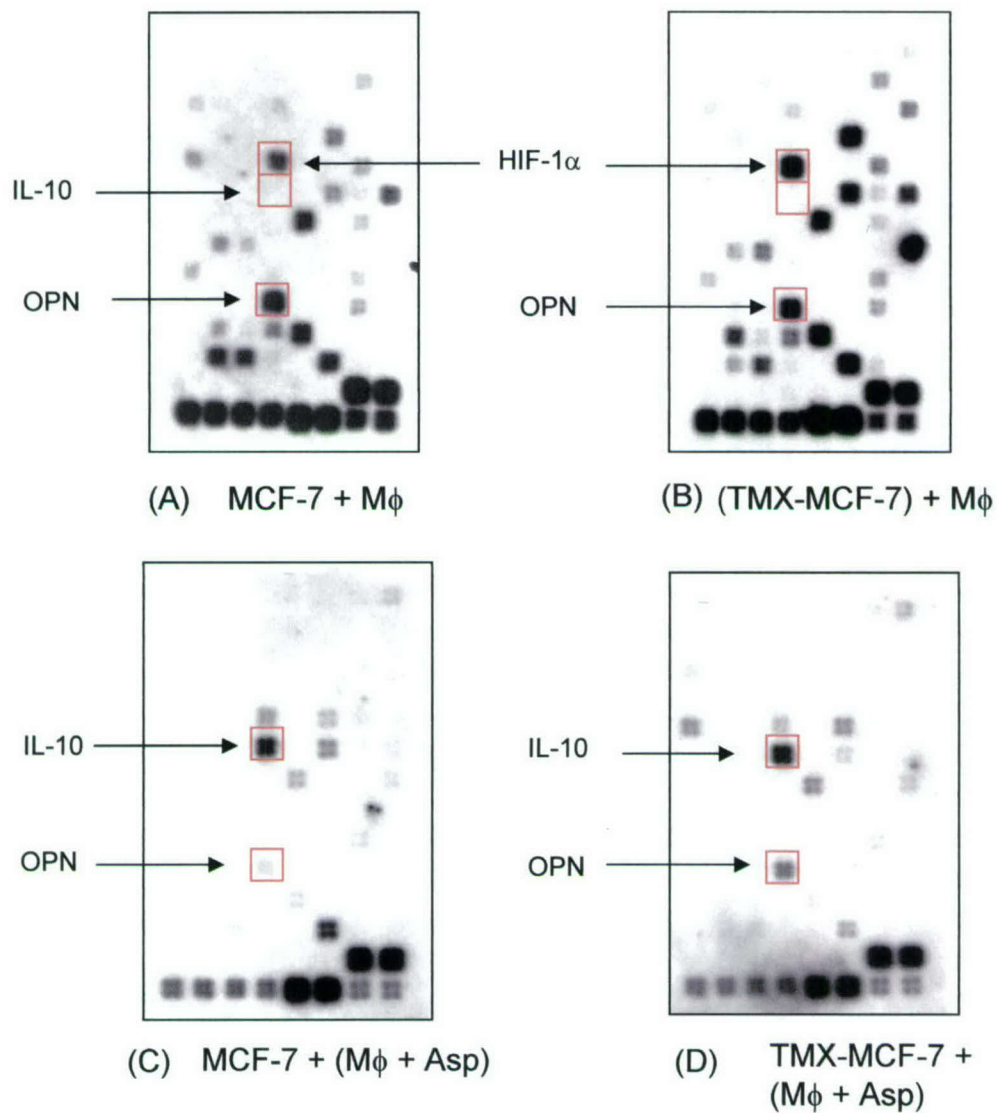
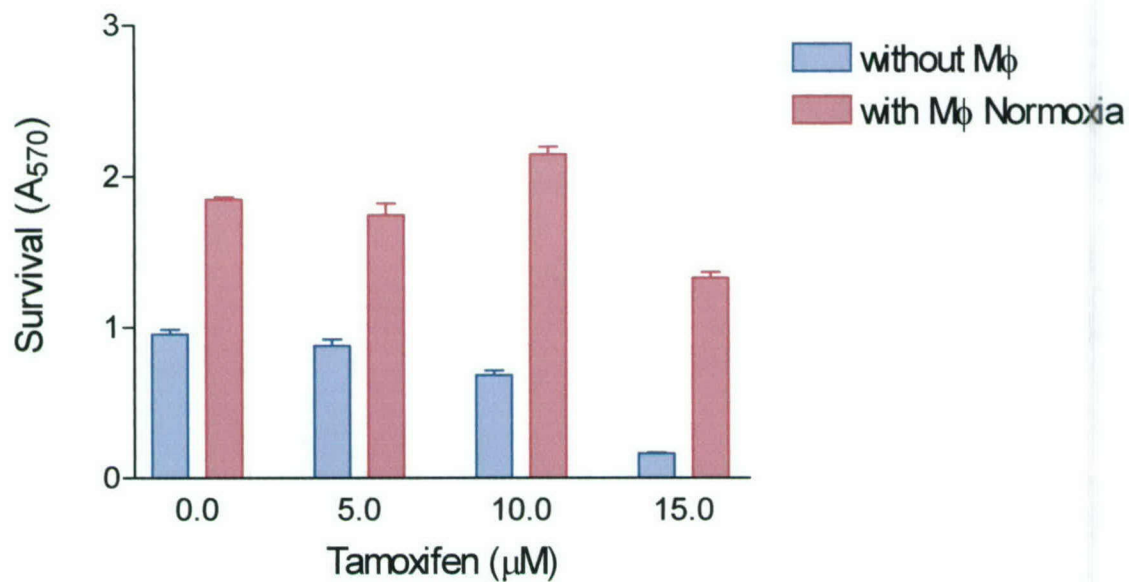


Fig. 6: Angiogenesis-related gene expression in MCF-7 cells under hypoxia. M ϕ , macrophages, TMX, tamoxifen, Asp, aspirin. Bryostatin 1-differentiated macrophages and MCF-7 cells were co-cultured in the upper and lower wells, respectively, of Costar TranswellTM chambers. The cells were separated by a membrane of pore size 3 μ m, which allowed passage of diffusible molecules, but not cells. After co-culture, mRNA was extracted and used for gene arrays.

A.



B.

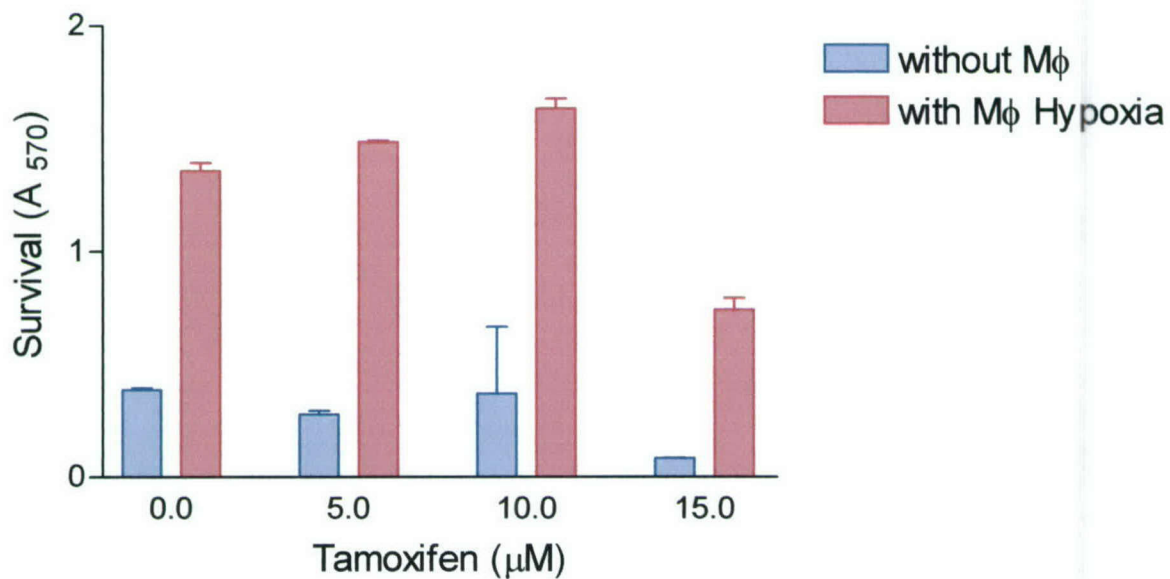


Fig. 7. Survival of tamoxifen-treated MCF-7 cells in the presence and absence of THP-1 macrophages under normoxia (A) and hypoxia (B). Cells were co-cultured as described previously. Proliferation and survival were measured by the MTT assay.

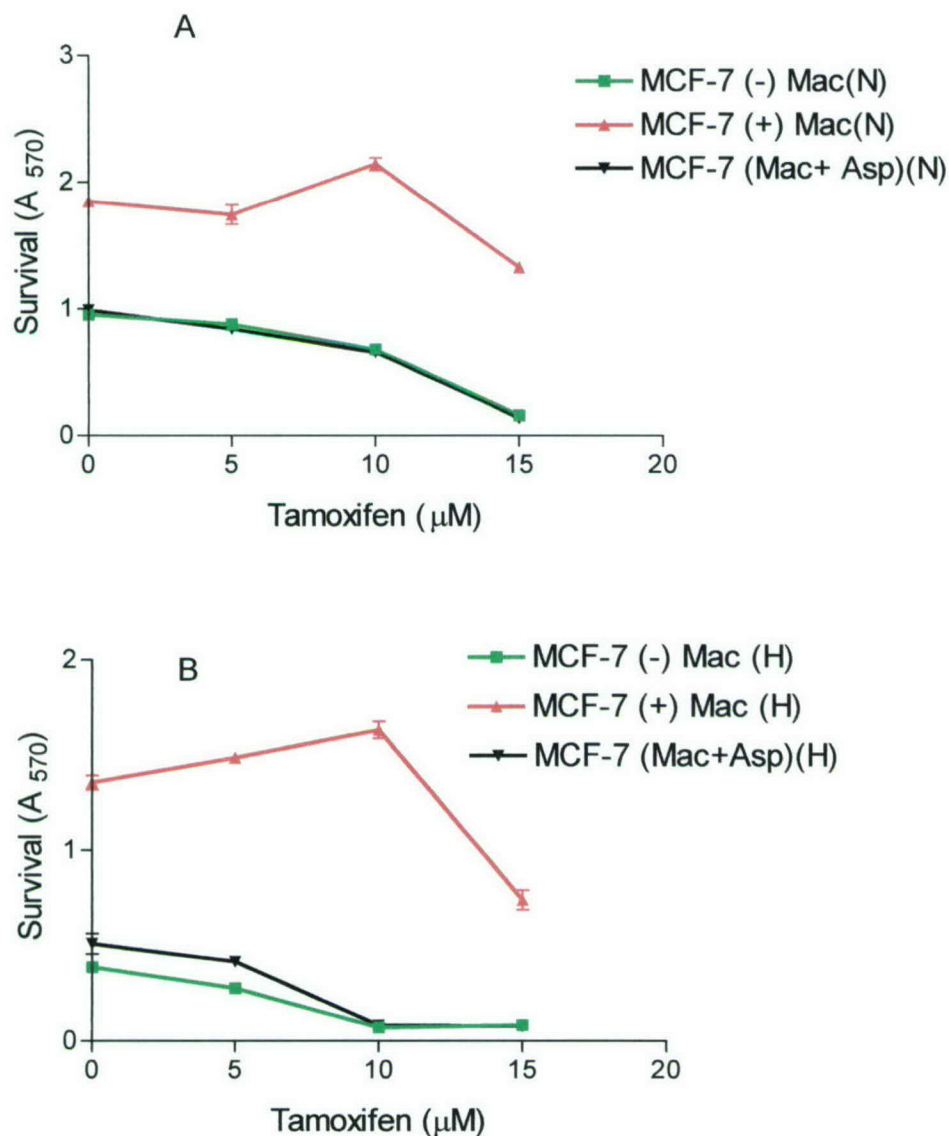


Fig. 8. Tamoxifen kills MCF-7 cells under normoxia (A) or hypoxia (B) in a dose-dependent manner (green). Macrophages protect MCF-7 cells against tamoxifen killing (red). Aspirin (1 mM) abrogates macrophage protection of MCF-7 (black). MCF-7 cells were treated with tamoxifen for 24 h; macrophages were treated with aspirin for 24 h; medium was changed and cells were co-cultured for 72 h. Survival was measured by MTT assay. Data points are means \pm SD (N = 3 replicates).

APPENDIX 5

Biosketch

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Bremner, Theodore A.		POSITION TITLE Associate Professor	
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Howard University, Washington, DC	B.S.	1962-1968	zoology
Howard University	M.S.	1968-1970	Zoology/genetics
Howard University	Ph.D.	1970-1972	Zoology/genetics

A. Positions and Honors.

1972-1978 Assistant professor, Department of Botany, Howard University
 1978-1979 Lecturer, Department of Zoology, Howard University
 1979-1980 Assistant professor, Dept. of Biology, Texas Southern University, Houston, TX
 1981-1985 Lecturer, Department of Zoology, Howard University
 1985-1992 Associate professor, Department of Zoology, Howard University
 1987-1990 Director, Honors Program, College of Liberal Arts (now College of Arts and Sciences), Howard University.
 1990-present Member, Basic Sciences Faculty, Howard University Cancer Center
 1992-present Associate professor, Department of Biology, Howard University
 1994-present Graduate associate professor, Graduate School, Howard University
 1996-1997 Howard Hughes Visiting Associate Professor of Molecular Biology (Research), Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI
 1997-2000 Adjunct associate professor of molecular biology, Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Other Experience and Professional Memberships

1974 Guest worker, Laboratory of Chemical Biology, National Institute of Arthritis, Metabolic and Digestive Diseases, NIH, Laboratory of Dr. Christian B. Anfinsen (summer).
 1976 Macy Scholar, Marine Biological Laboratory, Woods Hole, MA (summer)
 1994 Adjunct associate professor, Department of Genetics, College of Medicine, University of Illinois at Chicago (Summer).
 1999 Panelist, NSF Graduate Research Fellowship Program, Hyatt Regency Hotel, Crystal City, VA. Feb 4 to 7, 1999.
 1999 U.S. Army Medical Research and Materiel Command, 1999 Breast Cancer Research Program (BCRP) Scientific Peer Review, Panel: Cell Biology # 1, Sheraton Premiere Hotel, Tyson's Corner, Vienna VA, August 29-31, 1999.
 2000 Panelist, NSF Graduate Research Fellowship Program, Doubletree Hotel, Arlington, VA, Feb. 17 - 19, 2000.
 2001 Award in recognition of excellence in science and education, National Human Genome Research Institute, NIH, Bethesda, MD. 9 July 2001. (Ref. Jeff Witherly, Office of Science Education, NHGRI, E-mail: jlw@nhgri.nih.gov).
 2002-2004 Guest Researcher, Cancer Genetics Branch, National Human Genome Research Institute, NIH; Laboratory of Dr. Paul Meltzer, Head, Section on Molecular Genetics
 2002-present Member, Training Grant Advisory Committee ("Laboratory Research Training in Pediatric Oncology-Hematology" grant), Sidney Kimmel Comprehensive Cancer Center at Johns

- Hopkins.
Panelist (Scientific Reviewer), Susan G. Komen Foundation, Tumor Cell I Study Section, January 2004. (Contact: Deborah L. Price, Grant Review Specialist).
- 2004 Panelist (Scientific Reviewer), Department of Defense Ovarian Cancer Research Program, April 21 – 23, 2004, Landsdowne, VA. (Contact: Glacia Townsend)
- 2004 Panelist, Loan Repayment Program, NCMHD, NIH 16 – 18 May, 2004, Bethesda, MD. (Contact: Lorrita P. Watson)
- 2004* Panelist, Infectious Diseases Training Grant Study Section, Centers for Disease Control and Prevention, *August 9 – 13, 2004, Atlanta, GA.

Professional Memberships

- 1984-present American Association for the Advancement of Science
1996-present American Society for Cell Biology
2000-present Society for Leukocyte Biology
2002-present American Association for Cancer Research

B. Selected peer-reviewed publications (in chronological order).

1. Pipkin, S. B., and Bremner, T. A. (1970). Aberrant octanol dehydrogenase isozyme patterns in interspecific *Drosophila* hybrids. *J. Exp. Zool.* 175, 283-296.
2. Bremner, T. A. 1971. Octanol dehydrogenase: tissue distribution and function in *Drosophila metzii*. *Genetics* 68, Supplement No. 1/part 2: s7-s8.
3. Rowe, W. P., Hartley, J., and Bremner, T. A. (1972). Genetic mapping of a murine leukemia virus-inducing factor in AKR mice. *Science* 178, 860-862.
4. Pipkin, S. B., Chakrabartty, P. K., and T. A. Bremner. (1977). Location and regulation of *Drosophila* fumarase. *Journal of Heredity* 68, 245-252.
5. Bremner, T. A., Premkumar-Reddy, E., Nayar, K., and Kouri, R. (1978). Nucleoside phosphorylase 2 of mice. *Biochemical Genetics* 16, 1143-1152.
6. Collins, M. S., Wainer, I. W., and Bremner, T. A. (eds.) Science and the Question of Human Equality. American Association for the Advancement of Science, Selected Symposia Series. Westview Press, Frederick A. Praeger, Publisher. (1981).
7. Bremner, T. A., Anderson, M. D., Pope, G. J., and Anderson, R. M. (1983). Genetic polymorphism of amylase in three species of *Tribolium* (Coleoptera, Tenebrionidae). *Comparative Biochemistry and Physiology* 74B, 755-758.
8. Whiteside, C., Blackmon, R. H., and Bremner, T. A. (1983). Estrogen regulation of superoxide dismutase in normal rat mammary tissues and mammary tumors. *Biochemical and Biophysical Research Communications* 113, 883-887.
9. Soodeen, C. J., and Bremner, T. A. 1984. Biochemical heterogeneity of splenic purine nucleoside phosphorylase (NP-1A) from five species of wild mice. *Comparative Biochemistry and Physiology* 80B, 521-524.
10. Bremner, T. A., and Edwards, K. A. 1985. Inverse effects of ethidium bromide on superoxide dismutase and lactate dehydrogenase of *Artemia salina* embryos. *Journal of Experimental Zoology* 234, 1-5.
11. Bremner, T. A., Reid, Y. A., and Harrington, G. (1985). Superoxide dismutase and peroxidase are coordinately regulated in differentiated and transformed tissues of *Nicotiana tabacum*. *Differentiation* 28, 200-204.
12. Pope, G. J., Anderson, M. D., and Bremner, T. A. 1986. Constancy and divergence of amylase loci in four species of *Tribolium* (Coleoptera, Tenebrionidae). *Comparative Biochemistry and Physiology* 83B, 331-333.
13. Nayar, R., Lattimore, D., Sen, S., Huang, S., Holder, C., Nayar, S., and Bremner, T. A. 1992. Electrophoretic variants of glucose-phosphate isomerase in xenotrophic virus-producing mice. *Chromatin* 1, 157-167.
14. Emtage, M. A. and Bremner, T. A. (1993) Thermal regulation of active oxygen-scavenging enzymes in *Crithidia luciliae thermophila*. *Journal of Parasitology* 79, 809-814.

15. Asseffa, A., Dickson, L. A., Mohla, S., and Bremner, T. A. (1993) Phorbol myristate acetate-differentiated THP-1 cells display increased levels of MHC class I and class II mRNA and interferon- γ -inducible tumoricidal activity. *Oncology Research* 5, 11-18.
16. Bremner, T. A., D'Costa, N., Dickson, L. A., and Asseffa, A. (1996). A decrease in glucose 6-phosphate dehydrogenase activity and mRNA is an early event in phorbol ester-induced differentiation of THP-1 promonocytic leukemia cells. *Life Sciences* 58, 1015-1022.
17. Han, Z., Hendrickson, E.A., Bremner, T. A., and Wyche, J.H. (1997). A sequential two-step mechanism for the production of the mature p17:p12 form of caspase-3 in vitro. *Journal of Biological Chemistry* 272, 13432-13436.
18. Han, Z., Li, G., Bremner, T.A., Lange, T.S., Zhang, E.G., Jemmerson, R., Wyche, J.H., and Henderson, E.A. (1998). A cytosolic factor is required for mitochondrial cytochrome c efflux during apoptosis. *Cell Death and Differentiation* 5, 469-479.
19. Bremner, T. A., Chatterjee, D., Han, Z., Tsan, M.-F., and Wyche, J. H. (1999). THP-1 promonocytic leukemia cells express Fas ligand constitutively and kill Fas-positive Jurkat cells. *Leukemia Research* 23, 865-870.
20. Tsan, M.-F., White, J. E., Bremner, T. A., and Sacco, J. (2000). Resveratrol induces Fas signalling-independent apoptosis in THP-1 human monocytic leukaemia cells. *British Journal of Haematology* 109 (2), 405-412.
21. Chatterjee, D., Pantazis, P., Li, G., Bremner, T. A., Hendrickson, E. A., and Wyche, J. H. (2000). Susceptibility to apoptosis is restored in human leukemia HCW-2 cells following induction and stabilization of the apoptotic effector Bak. *Oncogene* 19, 4108-4116.
22. Smoot, D. T., Elliott, T. B., Verspaget, H. W., Jones, D., Allen, C. R., Vernon, K. G., Bremner, T., Kidd, L. C., Kim, K. S., Groupman, J. D., and Ashktorab, H. (2000). Influence of *Helicobacter pylori* on reactive oxygen-induced gastric epithelial cell injury. *Carcinogenesis* 21, 2091-2095.

C. Research Support.

Ongoing Research Support

DAMD17-02-1-0408 Bremner, (PI)

7/05/02-8/04/05

U.S Army Medical Research Acquisition Activity

Anti-estrogen Regulation of Macrophage Products That Influence Breast Cancer Cell Proliferation and Susceptibility to Apoptosis.

This study investigates inflammatory cytokine gene expression profiles induced in THP-1 macrophages by co-culture with breast cancer cells, as well as the ability of anti-estrogens to modulate reciprocal signaling between breast cancer cells and macrophages.

Role: PI

Wang, P (PI)

7/01/00-6/30/04

U.S Army Medical Research Acquisition Activity

A Training Program in Breast Cancer Research Using NMR Techniques.

This training program is designed to expose post-doctoral and graduate students in the physical sciences (Engineering) to NMR imaging of breast tumors.

Role: Investigator/mentor. To serve as a graduate research mentor; (2) to develop, test, and implement a cell biology training module for engineering graduate students to prepare them for participation in cancer research seminars and the graduate oncology course.

1U54CA91431-01. Adams-Campbell, L (PI)

5/01/01 - 4/30/06

NIH/NCI

Howard-Hopkins Cancer Partnership.

Project: Cancer Education Program. Theodore A. Bremner (Howard University Cancer Center) and Donald Coffey (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins), co-PIs.